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Responses of Microbiological Soil Properties to Intercropping at Different Planting Densities in an Acidic Andisol

Mauricio Schoebitz [1,*], Dalma Castillo [2], Milko Jorquera [3] and Antonio Roldan [4]

[1] Department of Soils and Natural Resources, Faculty of Agronomy, University of Concepción, 4030000 Concepción, Chile
[2] National Institute for Agriculture Research (INIA), Quilamapu, Chillán 3780000, Chile; dalma.castillo@inia.cl
[3] Scientific and Technological Bioresources Nucleus, University of La Frontera, Temuco 4780000, Chile; milko.jorquera@ufrontera.cl
[4] Center of Edafology and Applied Biology of Segura (CEBAS-CSIC), Department of Soil and Water Conservation, Espinardo Campus, 30100 Murcia, Spain; aroldan@cebas.csic.es
* Correspondence: mschoebitz@udec.cl

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Abstract: Intercropping could increase the capacity of crops to use soil resources. The aim of this study was to investigate the effects of lupin/wheat intercropping on soil properties, grain yield and nutrient uptake at different plant densities. Lupin and wheat were grown under field conditions as monocrops and intercrops. Soil nutrient availability and relative abundance of functional genes (*acdS*, *pho*D, *pho*C and *nif*H) were evaluated. The results obtained indicate that the cropping system had a significant effect ($p < 0.001$) on N and P availability. Lupin monocrop led to significantly higher N availability compared to intercrop. Intercropping resulted in significantly lower Olsen-P and K in soil concentrations compared to monocropping. No significant differences were observed in enzyme activity, except for phosphatase, which was 152% greater in the treatments at high plant density. Foliar nutrients were significantly higher in intercropping compared to monocropping. Acid phosphatase gene *pho*C was more abundant than the alkaline phosphatase gene *pho*D, which plays a more relevant role in acidic Andisols. The results confirm that N and P mobilization can improve nutrient absorption on wheat. When intercropped, lupin had positive effects on wheat due to its P mobilization capacity, while no effects were observed on lupin.

Keywords: enzyme activities; white lupin; wheat; facilitation; nutrient uptake; volcanic soil

1. Introduction

White lupin is a high-protein legume crop with the capacity to mobilize available nutrients in the soil, particularly P. Intercropping can have a positive effect on both component crops or even on subsequent crops [1]. Earlier studies [2] have described the capacity of white lupin to secrete organic acid and phosphatases from cluster roots (proteoid roots). Organic anion exudation increases mobilization of soluble P [3], while phosphatase secretion allows lupin to utilize both organic and inorganic P fractions in the soil. The enhanced capacity of lupin to secrete acid phosphatases under low-P conditions has been reported by Wasaki et al. [4]. This highlights the potential of this crop to improve P-availability for P-inefficient plant species when sharing rhizosphere functions in intercropping [5].

In acidic volcanic soils, P is bound to Al and Fe. Chilean volcanic soils present high amount of total P and organic matter, as well as high acidity level [6]. These soils, which are classified as Andisols, are the least extensive soil order [7] and occupy less than 1% of the earth's land surface or just below

963,000 km^2. In Chile, Andisols support the bulk of agricultural, pasture and forestry production, covering more than 5.3 × 10^6 ha, and representing 60% of the country's arable land [6].

Chile has the fastest growing production of lupin in the world. At present, there are about 25,000 ha under cultivation, reaching an annual production close to 40,000 tons. Most of this cultivated area is planted with locally-bred *Lupinus albus* L. A real estimate of maximum lupin production in Chile is 100,000 ha with an average yield of 4t ha^{-1} [8]. On the other hand, wheat is mainly grown under conventional farming. This method produces high yields, with an ideal quality for bread baking. To reach this, improved seed varieties need be used in order to obtain the full potential of a wheat variety [9]. However, this system requires farmers to use highly mechanized technological procedures for sole crops, with high requirements of agrochemicals [10].

Intercropping is one of the most widely used methods to increase crop production. There is evidence that growing two or more crops simultaneously in the same piece of land will produce a greater yield than a monoculture of any of the component crops, which is associated with interspecific interactions, including above- and belowground competition. In fact, belowground processes allow one crop to increase P and N availability for the other, improving yield of both species. However, the results of a study in maize intercropped with lupin showed that P facilitation was unidirectional as white lupin had a positive effect on maize, but not vice versa [5].

Associations with soil microorganisms allow plants to meet nutrient requirements. In fact, this interaction with microbial communities alters the habitat within the rhizosphere, which is the rooting zone where soil microorganisms quickly assimilate plant-derived C and compete with plants for soil nutrients [11]. On one hand, microorganisms can affect plant nutrition by influencing nutrient availability or by plant growth promotion [12]. On the other hand, plants can affect the diversity, activity and abundance of microorganisms by releasing diverse organic compounds (rhizodeposition). Rhizodeposition is an important energy source for the microbial production of extracellular enzymes that break down soil organic matter [13].

The aim of this experiment was to evaluate the effects of lupin/wheat intercropping on soil microbiological properties, grain yield and nutrient uptake at different plant densities in an acidic Andisol. As both crops are influenced by root interactions, the processes affecting nutrient uptake were also evaluated.

2. Materials and Methods

2.1. Site Description and Experimental Design

This study was conducted at the Santa Rosa Research Station of the National Agricultural Research Institute (INIA), Chillan, Chile (36°36′ S; 71°54′ W). The climate is classified as temperate Mediterranean, with an annual rainfall of 980 mm, with a potential evapotranspiration of 818 mm. Annual mean temperature is 13 °C, with an average temperature of 7 °C in July and 20 °C in January. Annual mean RH is 74% and the frost-free period is six months [14]. Prior to the initiation of this field experiment, corn was planted in 2017. Soil is classified as medial, amorphic, thermic Humic Haploxerands, originated from a volcanic ash-derived soil (Andisol) [7]. The chemical properties of the soil are as follows: pH 5.3, organic matter 12.4%, available N 17.8 mg kg^{-1}, available P 9.1 mg kg^{-1}, extractable K 77.6 mg kg^{-1}, aluminum saturation 1.11% and exchangeable Al 0.07 cmol$_c$ kg. Each treatment had three replicates with plot sizes of 3.2 × 4 m^2 randomly distributed in the field. For all the treatments, soil was amended with 25 kg N ha^{-1} year^{-1}, 125 kg P ha^{-1} year^{-1}, 100 kg K ha^{-1} year^{-1}, 50 kg Mg ha^{-1} year^{-1}, 45 kg S ha^{-1} year^{-1}, 20 kg Ca ha^{-1} year^{-1} and 0.5 kg B ha^{-1} year^{-1} and 0.5 kg Zn ha^{-1} year^{-1}. The plants were not irrigated during crop growth. Weeds were removed manually and no pesticides were applied. The cultivars used were Pantera-INIA (*T. aestivum*) and Alboroto-INIA (*L. albus*). Rizofix™ (Biogram S.A, Santiago, Chile) Gel inoculant (*Bradyrhizobium* sp. 1.0 × 10^9 colony forming unit mL^{-1}) was applied to lupin seeds prior to planting at the amount required to provide 1.0 × 10^7 colony forming unit of *Bradyrhizobium* per seed. Wheat and lupin were sown on 2 September 2018 and grown under three

cropping systems: wheat monocrop, lupin monocrop and wheat/lupin intercrop. Row spacing was 20 and 40 cm for wheat and lupin monoculture, respectively. For intercropping, both species were sown in the same row (row spacing of 40 cm). Row spacing was recommended according to the structure and development of the plants. It is the most suitable for the optimal growth of each crop. Three planting densities were used per cropping system; one was the recommended (medium) density and the other two were lower and higher densities. Wheat monocrop was planted at 150, 200 and 250 kg ha^{-1}; lupin monocrop was planted at 80, 140 and 200 kg ha^{-1}; and wheat/lupin intercrop was planted at 150 and 80 kg ha^{-1}(low density), 200 and 140 kg ha^{-1} (medium density), and 250 and 200 kg ha^{-1} (high density) for wheat and lupin, respectively (Figure 1). A completely randomized factorial design was used, with two factors and three replicates per treatment in a split plot arrangement. The first factor was cropping system (wheat monocrop, lupin monocrop and intercrop), while the second factor was plant density (low, medium and high).

Figure 1. Wheat intercropped with white lupin at low plant densities in an acidic Andisol.

2.2. Plant and Soil Sampling

Plant and soil samples were taken from three replicates of each crop at maturity stage (120 days after sowing) in February 2019. One linear meter per plot was sampled from the central row. In order to avoid edge-effects, one linear meter was delimited in the central area of each plot. Roots were gently removed from the soil, and the soil adhered to the roots was gently brushed and collected as rhizosphere soil from each plant. After this, each rhizosphere soil sample was sieved through a 2 mm mesh, and divided into two parts. One part was stored at 4 °C and then used to determine enzymatic activity and nutrients. The other part was stored at −20 °C for DNA extraction. In the monocrops, plants were randomly sampled, while wheat and lupin were sampled separately for the intercrop.

2.3. Plant and Soil Properties

Soil pH was measured in water 1:5 (w vol^{-1}). The available N, P and K were measured using the method described by Jackson [15] and Watanabe and Olsen [16]. Soil C biomass was evaluated by the substrate induced respiration method (SIR) [17]. The transformation of the amount of CO_2 emitted into microbial soil respiration used the equation developed by Anderson and Domsch [18]. Soil respiration and soil microbial biomass C (MBC) were determined with an automatic analyzer (µ-TRAC 4200, SY-LAB, Gerate GmbH, Neupurkersdorf, Austria). Dehydrogenase activity was determined according to Garcia et al. [19]. For this, 1 g of sample sieved to <2 mm at 60% of its field capacity was exposed to

0.2 mL of 0.4% INT (2-p-iod-ophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) in distilled water at 22 °C for 20 h in darkness. The iodonitrotetrazolium formazan (INTF) formed was extracted with 10 mL of methanol by vigorously shaking for 1 min and then filtered through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm. Urease and N-α-benzoyl-L-arginine amide (BAA) hydrolyzing protease activities were determined using 0.1 M phosphate buffer at pH 7; 1M urea and 0.03 M BAA were used as substrates, respectively. A volume of 2 mL of buffer and 0.5 mL of substrate were added to 0.5 g of soil sieved to <2 mm and then incubated at 30 °C (urease) or 40 °C (protease) for 90 min. Both enzyme activities were determined as the $NH4^+$ released in the hydrolysis reaction [20]. β-glucosidase determination is supported on the release and detection of p-nitrophenol (PNP). For this, 2 mL of 0.1M maleate buffer at pH 6.5 and a substrate consisting of 0.5 mL of *p*-nitrophenyl-β-D-glucopyranoside (PNG 0.05 M) were added to 0.5 g of soil sieved to <2 mm. Subsequently, the sample was incubated at 37 °C for 90 min. The reaction was stopped by the addition of tris-hydroxymethyl aminomethano (THAM) according to Tabatabai [21]. The amount of PNP was determined by spectrophotometry at 398 nm.

Acid phosphatase activity was determined using p-nitrophenyl phosphate disodium (PNPP 0.115 M) as substrate. An amount of 2 mL of 0.5 M sodium acetate buffer at pH 6 using acetic acid [22] and 0.5 mL of substrate were added to 0.5 g of soil sieved to <2 mm and then incubated at 37 °C for 90 min. The reaction was stopped by cooling at 0 °C for 10 min. Then 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH were added and the mixture was centrifuged at 4000 rev min^{-1} for 5 min. The p-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm [23]. Controls were conducted in the same way, but the substrate was added before the $CaCl_2$ and NaOH. Foliar concentrations of N and P were determined according to the methodology described by Sadzawka et al. [24]. Grain yield was evaluated by harvesting the whole plot (12.8 m^2) and expressed as kg ha^{-1}.

2.4. Counts of Functional Genes by Quantitative PCR (qPCR)

Bacterial functional genes involved in N_2 fixation (dinitrogenase reductase), alkaline phosphomonoesterase, acid phosphomonoesterase and 1-aminocyclopropane-l-carboxylate deaminase (ACCD) activities in rhizosphere soil samples were estimated by qPCR using the genes 16S rRNA, *nif*H, *pho*D, *pho*C and *acd*S, respectively. The ratio of gene target (*acd*S, *pho*D, *pho*C or *nif*H) in the bacterial community was estimated as relative abundance using absolute values of gene target in relation to total 16S rRNA gene counts. Total DNA was extracted from rhizosphere soil samples (0.25 g) by using DNeasy PowerSoil Kits (Qiagen, Inc., Hilden, Germany), according to the manufacturer's instructions. DNA concentrations were adjusted by dilution to 20 ng μL^{-1} and quality (ratio 260:280) was confirmed at ~1.8.

The quantitation of 16S rRNA genes was conducted by using the mitochondria- and chloroplast-excluding primer set 799f (5′-AAC MGG ATT AGA TAC CCK G-3′) and 1115r (5′-AGG GTT GCG CTC GTT G-3′) described by Shade et al. [25]. The quantitation of functional genes was achieved by using the following primer sets: (1) nifH-g1-forB (5′-GGT TGT GAC CCG AAA GCT GA-3′) and nifH-g1-rev (5′-GCG TAC ATG GCC ATC ATC TC-3′) for *nif*H gene (N_2-fixing; [26]), (2) ALPS-F730 (5′- CAG TGG GAC GAC CAC GAG GT-3′) and ALPS-R1101 (5′-GAG GCC GAT CGG CAT GTC G-3′) for *pho*D gene (alkaline phosphatase; [27]), (3) phoc-A-F1(5′-CGG CTC CTA TCC GTC CGG-3′) and phoc-A-R1 (5′CAA CAT CGC TTT GCC AGT G-3′) for *pho*C gene (acid phosphatase; [28]) and (4) acdSf3 (5′-ATC GGC GGC ATC CAG WSN AAY CAN AC-3′) and acdSr3 (5′-GTG CAT CGA CTT GCC CTC RTA NAC NGG RT-3′) for *acd*S gene (ACCD); [29].

All qPCR reactions were performed using PowerUp™ SYBR® Green Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), while PCR conditions were set according to the manufacturer's instructions: 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. PCR reactions were performed in a StepOnePlus™ Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA).

Gene copies were estimated by using standard curves prepared with synthetic dsDNA ultramers (Integrated DNA Technologies, Inc., Coraville, IA, USA) of the 16S rRNA gene from *Azospirillum picis* (NCBI accession no. AM922283), *nif*H gene from *Azospirillum brasilense* Sp7 (NCBI accession no. X51500), *pho*D gene from uncultured bacterium (NCBI accession no. MF488694.1), *pho*C gene from *Escherichia blattae* (NCBI accession no. AB020481.1), and *acd*S gene from *Pseudomonas putida* (NCBI accession no. AY823987), respectively.

2.5. Statistical Analysis

The experiment design was a two-factor randomized block in a split-plot arrangement with three replicates per treatment. The effects of cropping system, plant density and their interactions were analyzed by a two-way ANOVA. Post-hoc mean separation was performed by Duncan's multiple range test at $p \leq 0.05$ by using the SPSS software version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil Nutrients

Soil chemical properties of wheat and lupin monocrops, and wheat intercropped with lupin at different plant densities are described in Table 1. Values of pH did not vary significantly between monocrops and wheat/lupin intercrop at the three plant densities studied. A significant interaction ($p < 0.001$) was found in nitrate content, particularly in lupin monoculture, which recorded the highest nitrate level in the soil. At low plant density, rhizosphere nitrate concentration recorded by lupin monoculture was 2.5- and 2.7-fold higher than values observed in wheat monocrop and wheat intercropped with lupin, respectively. Regarding the factorial analysis, cropping system (CS), plant density (PD) and CS x PD interactions were significant (Table 1). As for NH_4, there were no significant differences among plant density levels in each one of the treatments. Cropping system resulted in significant differences ($p < 0.001$) in N availability in the soil. Lupin monocrop recorded significantly higher N availability than wheat monocrop and wheat/lupin intercrop.

Olsen-P levels were significantly lower in wheat intercropped with lupin compared to values observed in wheat monocrops. In addition, significant differences were found in soil N availability ($p < 0.001$) among cropping systems. A similar trend was observed in terms of K available in wheat/lupin intercrop as levels were significantly lower than those in wheat monocrop. The ANOVA showed a significant effect of cropping system, while plant density did not affect K availability (Table 1).

3.2. Microbiological Soil Properties

With regard to soil enzyme activities, intercropping significantly increased in acid phosphatase compared to wheat monocropping. Soil phosphatase levels observed in intercropping were 4.6-, 8.6- and 2.4-fold higher than those recorded in wheat monocrops at low, medium and high plant density, respectively. However, phosphatase content did not record a significant increase in lupin monoculture compared to wheat/lupin intercropping, but it resulted in a significant increase in phosphatase activity in rhizosphere soil (152% greater than wheat/lupin intercropping at high plant density). The factorial analysis showed that cropping system (CS), plant density (PD) and CS × PD interactions were not significant in terms of microbial biomass, soil basal respiration, urease, protease, β-glucosidase and dehydrogenase activities (Table 2).

Table 1. Soil chemical properties in response to monocropping and intercropping at different plant densities under field conditions.

Cropping System	Plant Density (kg ha^{-1})	pH	NO_3 (mg kg^{-1})	NH_4 (mg kg^{-1})	N Available (mg kg^{-1})	P Olsen (mg kg^{-1})	K Available (mg kg^{-1})
Wheat	L	5.3 ± 0.0	5.2 ± 0.4 ab	5.6 ± 0.6 ab	10.8 ± 0.7 ab	12.0 ± 1.0 cd	166 ± 11 c
	M	5.0 ± 0.3	4.4 ± 0.4 a	4.0 ± 0.4 a	8.4 ± 0.4 a	11.7 ± 0.7 bcd	144 ± 26 bc
	H	5.3 ± 0.0	5.1 ± 0.1 ab	5.1 ± 0.4 a	10.1 ± 0.5 ab	13.2 ± 2.1 d	165 ± 23 c
Lupin	L	5.4 ± 0.1	13.0 ± 0.5 d	7.8 ±0.7 c	20.8 ± 0.9 e	9.8 ± 0.2 abc	95 ± 15 a
	M	5.3 ± 0.0	10.9 ± 0.9 c	7.0 ± 1.0 bc	17.9 ± 1.8 d	9.0 ± 0.3 ab	107 ± 8 ab
	H	5.3 ± 0.0	7.1 ± 1.3 b	7.3 ± 0.4 bc	14.4 ± 1.2 c	9.5 ± 0.5 abc	83 ± 9 a
Wheat + Lupin	L	5.3 ± 0.1	4.8 ± 0.1 a	5.7 ± 0.3 ab	10.5 ± 0.6 ab	8.0 ± 0.4 a	81 ± 10 a
	M	5.3 ± 0.0	6.6 ± 0.6 ab	7.2 ± 0.3 bc	13.8 ± 0.8 c	8.4 ± 1.0 a	90 ± 1 a
	H	5.4 ± 0.0	4.8 ± 0.4 a	8.0 ± 0.5 c	12.7 ± 0.6 bc	8.4 ± 0.3 a	85 ± 6 a
Anova, P values							
Cropping system		0.191	<0.001	<0.001	<0.001	<0.001	<0.001
Plant density		0.265	0.003	0.340	0.130	0.661	0.971
CS ×PD		0.785	0.001	0.053	0.001	0.845	0.557

Values are means of three replicates. Mean ± standard error. Significant difference according to the Duncan test at $P < 0.05$ levels was indicated by different letters. Low (L) plant density: 150 kg ha^{-1}; medium (M) plant density: 200 kg ha^{-1}; high (H) plant density 250 kg ha^{-1}; (wheat). Low (L) plant density: 80 kg ha^{-1}; medium (M) plant density: 140 kg ha^{-1}, high (H) plant density 200 kg ha^{-1} (lupin).

Table 2. Soil microbiological properties and enzyme activities in response to monocropping and intercropping at different plant densities under field conditions.

Cropping System	Plant Density (kg ha^{-1})	Phosphatase (μmol PNP g^{-1}h^{-1})	Urease (μmol NH_4^+ g^{-1}h^{-1})	Protease (μmol NH_4^+ g^{-1}h^{-1})	Dehydrogenase (μg INTF g^{-1})	β-glucosidase (μmol PNP g^{-1}h^{-1})	Soil Basal Respiration (CO_2 h^{-1} Kg^{-1})	Microbial Biomass C (mg Kg^{-1})
Wheat	L	0.5 ± 0.07 a	2.3 ± 0.10	1.1 ± 0.04	22 ± 1.0	0.36 ± 0.01	12.0 ± 0.9	1495 ± 192
	M	0.3 ± 0.11 a	1.9 ± 0.04	0.9 ± 0.13	19 ± 0.9	0.30 ± 0.04	11.1 ± 0.4	1495 ± 141
	H	0.7 ± 0.0 a	2.1 ± 0.15	0.8 ± 0.14	22 ± 2.4	0.32 ± 0.05	11.8 ± 0.6	1548 ± 175
Lupin	L	2.2 ± 0.17 bc	2.0 ± 0.04	0.7 ±0.10	23 ± 2.0	0.33 ± 0.02	12.0 ± 0.8	1575 ± 175
	M	2.9 ± 0.16 d	2.0 ± 0.14	0.8 ± 0.11	23 ± 0.6	0.31 ± 0.03	13.4 ± 0.2	1575 ± 175
	H	2.6 ± 0.29 cd	2.1 ± 0.11	0.9 ± 0.08	24 ± 1.6	0.30 ± 0.04	14.1 ± 0.9	1842 ± 46
Wheat + Lupin	L	2.3 ± 0.21 cd	2.0 ± 0.19	1.0 ± 0.05	22 ± 0.8	0.33 ± 0.01	13.4 ± 1.0	1682 ± 45
	M	2.6 ± 0.41 cd	2.1 ± 0.18	0.9 ± 0.05	22 ± 2.1	0.32 ± 0.01	12.0 ± 0.6	1642 ± 23
	H	1.7 ± 0.23 b	2.0 ± 0.10	0.9 ± 0.10	24 ± 1.6	0.31 ± 0.03	13.3 ± 0.9	1782 ± 46
Anova, P values								
Cropping system		<0.001	0.654	0.114	0.082	0.881	0.051	0.216
Plant density		0.125	0.698	0.435	0.323	0.428	0.313	0.336
CS × PD		0.019	0.211	0.307	0.702	0.943	0.332	0.903

Values are means of three replicates. Mean ± standard error. Significant difference according to the Duncan test at $P < 0.05$ levels was indicated by different letters. Low (L) plant density: 150 kg ha^{-1}; medium (M) plant density: 200 kg ha^{-1}; high (H) plant density 250 kg ha^{-1}; (wheat). Low (L) plant density: 80 kg ha^{-1}; medium (M) plant density: 140 kg ha^{-1}, high (H) plant density 200 kg ha^{-1} (lupin). PNP: p-nitrophenyl phosphate. INTF: iodonitrotetrazolium formazan.

3.3. Nutrient Uptake and Grain Yield

Total N concentration showed a significant increase in wheat/lupin intercropping at the three plant densities studied. Values of wheat foliar N were 36%, 21% and 40% higher in intercropping than in wheat monocropping at low, medium and high plant density, respectively (Table 3).

Table 3. Grain yields, foliar N and P of monocropping (sole wheat and sole lupin) and wheat/white lupin intercropping at different plant densities under field conditions.

	Cropping	Plant Densiy	N%	P%	Grain Yield (kg ha^{-1})
Wheat	Monocrop	L	1.4 ± 0.2 b	0.09 ± 0.02 ab	5331 ± 193 c
		M	1.4 ± 0.1 b	0.10 ± 0.01 b	6510 ± 329 d
		H	1.0 ± 0.1 a	0.07 ± 0.01 a	5733 ± 360 cd
	Intercrop	L	1.9 ± 0.1 d	0.14 ± 0.01 c	2529 ± 395 b
		M	1.7 ± 0.0 cd	0.12 ± 0.01 bc	1593 ± 189 a
		H	1.4 ± 0.1 bc	0.11 ± 0.01 bc	1522 ± 79 a
	Cropping (C)		<0.001	<0.001	<0.001
	Plant density (PD)		0.002	0.047	0.332
	C × PD		0.756	0.428	0.08
Lupin	Monocrop	L	4.2 ± 0.3	0.25 ± 0.01	4039 ± 215 b
		M	4.5 ± 0.1	0.27 ± 0.01	4630 ± 329 bc
		H	4.0 ± 0.2	0.25 ± 0.0	5105 ± 117 c
	Intercrop	L	4.3 ± 0.2	0.25 ± 0.01	3099 ± 138 a
		M	4.0 ± 0.1	0.26 ± 0.01	4314 ± 414 bc
		H	4.4 ± 0.0	0.26 ± 0.0	4315 ± 231 bc
	Cropping (C)		0.939	0.856	0.008
	Plant density (PD)		0.991	0.421	0.002
	C × PD		0.057	0.314	0.484

Values are means of three replicates. Mean ± standard error. Significant difference according to the Duncan test at $P < 0.05$ levels was indicated by different letters. Low (L) plant density: 150 kg ha^{-1}; medium (M) plant density: 200 kg ha^{-1}; high (H) plant density 250 kg ha^{-1}; (wheat). Low (L) plant density: 80 kg ha $^{-1}$; medium (M) plant density: 140 kg ha^{-1}, high (H) plant density 200 kg ha^{-1} (lupin).

No treatment significantly affected total N and P nutrient content in lupin monocrop or intercrop (Table 3). The cropping system had significant effect ($p < 0.001$) on N and P levels in wheat plants. In addition, significant differences were found in wheat foliar N (0.002) among plant densities.

P concentration in wheat increased significantly with intercropping at low (56%) and high (57%) plant density. Regarding wheat grain yield, the post-hoc test showed a significant decrease in grain yield in intercropping at low, medium and high plant density (53%, 76% and 73%, respectively), and a significant decrease in lupin grain yield only at low plant density in the same cropping system. No significant differences were observed in terms of lupin grain yield between monocropping and intercropping at medium and high plant densities.

3.4. Relative Abundance of Bacterial Functional Genes

Regardless of the treatment, relative abundance of rhizobacterial populations was lower in the alkaline phosphomonoesterase gene (*pho*D) compared to the acid phosphomonoesterase gene (*pho*C) (Figure 2). In wheat monocropping, relative abundance of *pho*C and dinitrogenase reductase (*nif*H) genes was significantly higher at higher plant densities, but this pattern was not observed in lupin monocropping and intercropping. Interestingly, rhizobacterial populations in lupin monoculture and intercropped with wheat showed a significantly higher relative abundance of 1-aminocyclopropane-l-carboxylate deaminase (ACCD) gene compared to rhizobacterial populations found in wheat monocrops. For all the genes, the amplification efficiency values ranged from 91.3% to 110.1% and the linear fit R^2 was greater than 0.966.

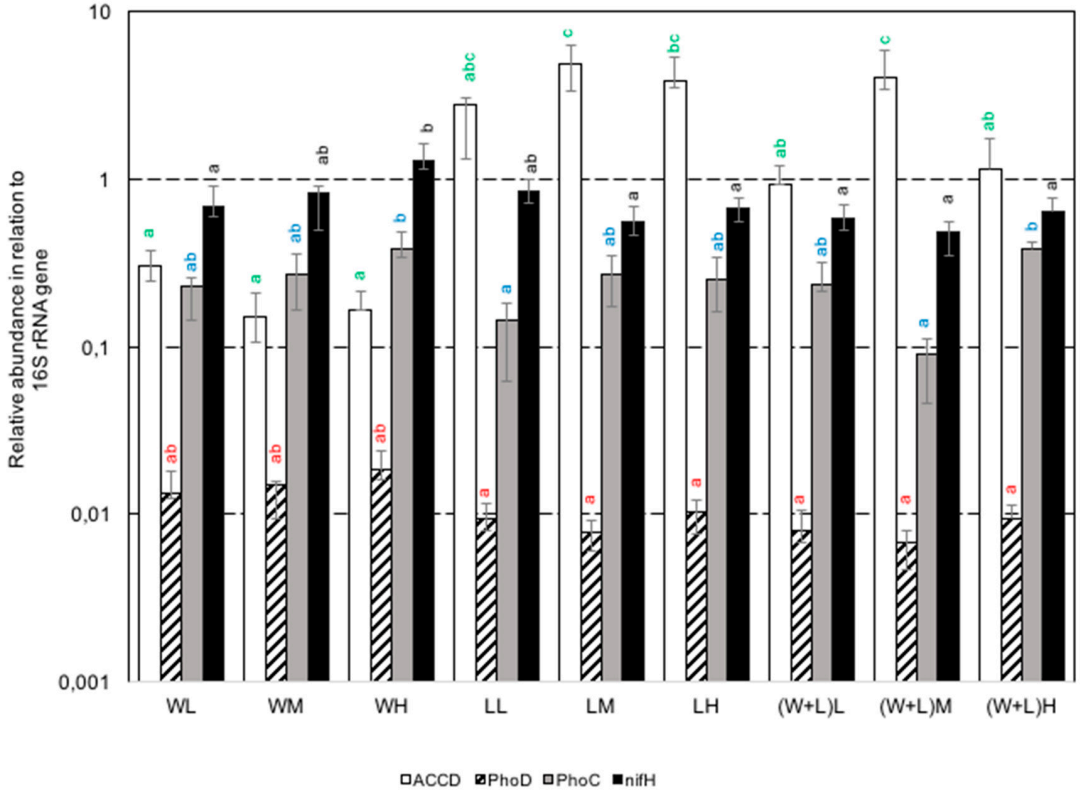

Figure 2. Relative abundance of functional genes in relation to the16S rRNA gene determined by quantitative PCR (qPCR). *acd*S: 1-aminocyclopropane-l-carboxylate deaminase (ACCD); *pho*D: alkaline phosphomonoesterase, *pho*C: non-specific acid phosphomonoesterase; *nif*H: N_2 fixation. Error bars represent the standard error. Different letters of the same color indicate significant differences according to Duncan test ($P < 0.05$). WL: wheat at low plant density (PD); WM: wheat at medium PD; WH: wheat at high PD; LL: lupin at low PD; LM: lupin at medium PD; LH: lupin at high SD; (W + L)L: intercropping at low PD; (W + L)M: intercropping at medium PD and (W + L)H: intercropping at high PD.

4. Discussion

Plant interaction can increase the economic and biological yield of wheat crop, mainly through positive influence where a crop can modify the environment of its roots [30]. The benefits of intercropping are manifold. Increased yields allow for more energy production and higher profits by farmers [31]. Most studies on cereal/legume intercropping implicitly assume that the cereal crop will benefit from the legume crop because legumes are known to excrete larger amounts of protons, carboxylates, phosphatases [32] and organic acids in their rhizosphere (citrate, malate, succinate and fumarate) [33]. Dissanayaka et al. [5] found that maize/lupin intercropping is unidirectional since lupin increases soil nutrient availability and thus nutrient uptake in maize, while there is no benefit to lupin. Our results confirm these findings since intercropping had no effect on white lupin in terms of soil nutrient availability or microbiological soil properties.

Wheat intercropped with lupin results in competition between the aerial parts of the plant and also between the underground parts. It has been described that plant density influences root distribution, N use and grain yields of *Vicia faba* intercropped with oil crops [34]. In our experiment, plant density had no effects on microbiological soil properties or grain yield. However, soil nitrate increased in lupin monocrop and N foliar content in wheat/lupin intercrop at low and medium plant density. This suggests that the effects of plant density on N content may become more pronounced if the root system is not dense. The results obtained in our study show that high competition for plant nutrients had no effects on grain yield at high plant density in the acidic soil. Similar results were found in a

study in maize by Xia et al. [35], who reported that high plant density did not result in higher grain yield in three different maize/peanut intercropping experimental sites.

Our results show that there were differences in soil nitrate between monocropping and intercropping, but with no complementary effect by intercropping. White lupin is a leguminous plant, nodulated early by *Bradyrhizobium lupini.* Lupin has the ability to increase soil N through symbiotic fixation [36]. Soil nitrate availability in lupin monocrop was higher than that in wheat monocrop and wheat/lupin intercrop, which suggests that white lupin alone increases nitrate content. The decrease in inorganic N in the rhizosphere observed in the intercropping experimental sites was probably due to a consequence of wheat root uptake. Intercropping reduced soil Olsen-P and available K compared to the monocrops. This could be explained because P acquisition by intercropping was significantly greater than wheat and lupin sole crops. Similar results were reported in maize intercropped with faba bean, soybean and chickpea in field experiments conducted in a fertile soil [35,37].

Microbial enzyme activities affected by the type of cropping system used include dehydrogenase activity, which is relevant for the decomposition of soil organic matter, N dynamics, and can be used as a sensitive marker of soil degradation and soil microbiological properties [19,38,39]. In this work, urease activity was not influenced by cropping system or plant density. In contrast, a study conducted in maize/soybean intercropping [40] showed that this system increases urease, protease and soil nitrate reductase activity under field conditions. Our results obtained herein, suggesting that wheat/lupin intercropping has no clear effects on soil urease, protease, β-glucosidase and dehydrogenase activity. Similar results were also reported by Wang et al. [37], who conducted a study in maize intercropped with faba bean, and found that intercropping did not affect enzyme activity over a period of two years in Orthic Antrosols under field conditions, except for soil acid phosphatase activity; this was higher in maize/legume intercropping compared to values observed in the corresponding monocrops at 40 kg ha^{-1} P.

Our results demonstrated that wheat/lupin intercropping and lupin monocropping enhanced soil acid phosphatase activity compared to wheat monocropping, which indicates that lupin may utilize more soil organic P. Soil extractable P was strongly related to acid phosphatase in soil, because phosphatase activity was associated with soil organic P mobilization [41]. It has been determined that the roots of lupin exudate large amounts of phosphatase into their rhizosphere [4], which might explain the enhanced acid phosphatase activity observed in lupin monocropping and wheat/lupin intercropping. Wasaki et al. [42] have reported that white lupin secretes acid phosphatase from the whole root system not only in cluster roots. However, cluster roots secreted higher amounts of phosphatase in P-deficient soil conditions. These results support that cluster root formation plays a significant role in the acquisition of phosphorus from soils.

Different soil management practices and intercropping can increase soil microbiological properties and enzyme activity [43,44]. The comparison between microbial quantity and diversity could be useful to understand how rhizosphere communities influence crop yield under different agricultural practices. It has been demonstrated that the increase in MBC on intercropping occurs due to higher diversity of rhizodeposits in the rhizosphere of intercropped species. In this sense, Tang et al. [43] reported no significant changes in bulk soil for MBC compared to rhizosphere soil of sole crops under field conditions, while MBC was significantly higher in the rhizosphere of intercrops, with the highest values being recorded by chickpea monocrop and lentil intercropped with wheat. In our study, no significant changes were found for MBC and dehydrogenase activity in the intercropping treatments. Similar results were found by Song et al. [44], who reported that intercropping decreased MBC in the rhizosphere of faba bean in the first year of the field experiment. This suggests that the effects of intercropping on microbial properties may become more pronounced if the cropping system is maintained over several years. The fact that crop residues are incorporated into the soil after harvest may also result in stronger effects. In this sense, the effects of wheat/white lupin intercropping were not significant in an acidic Andisol in our study, which is in agreement with the findings of Dissanayaka et al. [5]. They observed significant effects on maize intercropped with lupin in a Regosol,

but not in an Andisol. Their results showed higher organic matter content in the volcanic soil with acidic pH conditions, with dominant presence of allophane, active aluminum and high P adsorption, which might have led to slight changes in microbial properties in monocropping and intercropping (except for acid phosphatase activity). Similar results were also found in a study conducted by Kunito et al. [45] in an Andisol. These authors reported an inhibition of enzyme activities (β-glucosidase, polyphenol oxidase and L- asparaginase) and microbial biomass. Kunito et al. [45] showed that the results of the enzyme activities in acidic Andisol imply that Al toxicity and low pH could reduce soil microbial functions. Unlike other enzymes, acid phosphatase activity did not decrease in the acidic Andisol. The higher activity was caused by a production of acid phosphatase, which is needed to obtain P from organic matter in acidic soils. Our study demonstrated that monocropping and intercropping presented low pH (5.0–5.3). Acidic conditions restrained soil enzyme activities (urease, protease, dehydrogenase and β-glucosidase), soil basal respiration and microbial biomass. This inhibition may be due to the depressed amount of enzyme synthesis and/or decreased populations of microorganisms in acidic soil. Similar results were found by Kunito et al. [45] evaluated in Andisol and Inceptisols.

A field trial conducted by Xia et al. [35] reported a 24% increase in grain yield in maize intercropped with faba bean compared to the weighted means of the corresponding monocrops. Similar results (23%) were found in another study of maize/faba bean intercropping [46]. Conversely, the present study found that intercropping had no significant effects on total grain yield compared to monocropping. Over a period of one year, wheat intercropped grain yields were lower compared to monocropping. The fact that intercropping resulted in a reduction in wheat grain yield can be explained by low soil fertility, number of wheat plants per plot and competitive ability. In terms of low soil fertility, acidic conditions would explain high fixation of applied P in less labile pools. Besides, Andisols are rich in allophones containing active aluminum and show high retention of P, which implies that fertilizer requirements are particularly low. Low fertility conditions directly affect grain yield of wheat as this is a non-legume and a non-P-mobilizing crop. Regarding the number of wheat plants per plot, row spacing was 20 and 40 cm for wheat and lupin monocrops, respectively. In intercropping treatments, both crops were sown in the same row (40 cm row spacing), resulting in a 41% decrease in plant number per plot and a 53% reduction in total biomass, considering 1 lineal meter per plot (data not shown). Another study showed that barley and wheat had different responses in yield and competitive ability. Wheat suffers more competition from a legume crop than barley. Barley allowed for more rapid establishment and earlier rate of leaf production, leading to the observed dominance exerted by this species when intercropped [30]. In this study, we found a significant decrease in wheat total grain yield in intercropping, probably because lupin competed for the aerial space more than wheat.

Numerous studies have also indicated that intercropping facilitates productivity and nutrient acquisition compared to monocrops [30,37,47]. Thus, intercropping significantly removes more nutrients from the soil than monocrops. For instance, intercropped wheat showed greater N uptake (by 21–40%) than monocrops and a 56–57% increase in wheat P uptake in intercropping compared to monocropping. The results obtained herein confirmed that intercropping removes more N in wheat-based systems than in monocrops. There are similar results for N acquisition in maize/faba bean, barley/maize [48] and maize/faba bean, maize/soybean, maize/chickpea and maize/turnip intercropping [37]. Therefore, it is important to know how soil properties change with higher yields and nutrient removals from soil in intercropping when compared to monocrops.

Regardless of the cropping system, abundance of functional genes within the total bacterial community, measured in relation to the 16S rRNA gene, showed higher abundance of acid phosphomonoesterase genes (*pho*C) compared to alkaline phosphomonoesteraseb (*pho*D). This result suggests that bacterial acid phosphatase activity has an important function in rhizobacterial populations from Chilean Andisols, which are characterized by high contents of organic P and low soil pH levels [6]. In this sense, previous findings have also described that bacterial community compositions and phosphatase gene compositions in grassland and forest were mainly influenced by soil pH [49].

Interestingly, higher abundances of *acd*S gene were observed in lupin rhizospheres compared with sole wheat rhizospheres as a result of selective recruitment of microorganisms by the lupin plants. The gene *acd*S has been widely used as a molecular maker of ACCD activity in rhizobacteria, which provides a higher tolerance to abiotic stress in plants [50]. Diverse studies have explained the differences in bacterial community composition among plant species; each plant species releases root exudates to recruit specific bacterial populations, which can provide higher nutrient supply to the plants [51]. However, higher occurrence of *acd*S gene cannot be explained by a higher stress level in lupin based on data measured in this study.

The results obtained confirmed that N and P mobilization of lupin from soluble N and P pools can increase nutrient acquisition efficiency in an acidic Chilean Andisol with low fertility. Nutrient facilitation is unidirectional. Thus, intercropping results in a positive effect of lupin on wheat crop, but not vice versa. Enhanced N uptake by wheat intercropped with lupin is dependent on plant density.

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References

1. Lambers, H.; Clements, J.C.; Nelson, M.N. How a Phosphorus-Acquisition Strategy Based on Carboxylate Exudation Powers the Success and Agronomic Potential of Lupines (Lupinus, Fabaceae). *Am. J. Bot.* **2013**, *100*, 263–288. [CrossRef] [PubMed]
2. Shane, M.W.; Lambers, H. Cluster roots: A curiosity in context. *Plant Soil* **2005**, *274*, 101–125. [CrossRef]
3. Mora, M.D.; Demanet, R.; Acuna, J.J.; Viscardi, S.; Jorquera, M.; Rengel, Z.; Duran, P. Aluminum-tolerant bacteria improve the plant growth and phosphorus content in ryegrass grown in a volcanic soil amended with cattle dung manure. *Appl. Soil Ecol.* **2017**, *115*, 19–26. [CrossRef]
4. Wasaki, J.; Yamamura, T.; Shinano, T.; Osaki, M. Secreted acid phosphatase is expressed in cluster roots of lupin in response to phosphorus deficiency. *Plant Soil* **2003**, *248*, 129–136. [CrossRef]
5. Dissanayaka, D.M.S.B.; Maruyama, H.; Masuda, G.; Wasaki, J. Interspecific facilitation of P acquisition in intercropping of maize with white lupin in two contrasting soils as influenced by different rates and forms of P supply. *Plant Soil* **2015**, *390*, 223–236. [CrossRef]
6. Mora, M.D.; Rosas, A.; Ribera, A.; Rengel, Z. Differential tolerance to Mn toxicity in perennial ryegrass genotypes: Involvement of antioxidative enzymes and root exudation of carboxylates. *Plant Soil* **2009**, *320*, 79–89. [CrossRef]
7. Staff, S.S. *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*; US Government Printing Office: Washington, DC, USA, 2006.
8. Information Resource Portal for Lupin. Available online: lupins.org/lupins (accessed on 16 September 2019).
9. Mellado, M. *El Trigo en Chile, Colección Libros INIA Nº 21*; Instituto de Investigaciones Agropecuarias, Centro Regional de Investigación (CRI) Quilamapu: Chillán, Chile, 2007.
10. Montalba, R. *Cambio Técnico Agrario y Sostenibilidad de los Agroecosistemas. Guía Curso "Agroecología. y Desarrollo Rural Sostenible". Unidad 1*; Universidad de La Frontera: Temuco, Chile, 2009; 93p.
11. Dijkstra, F.A.; Carrillo, Y.; Pendall, E.; Morgan, J.A. Rhizosphere priming: A nutrient perspective. *Front. Microbiol.* **2013**, *4*, 216. [CrossRef]
12. Dodd, I.C.; Ruiz-Lozano, J.M. Microbial enhancement of crop resource use efficiency. *Curr. Opin. Biotechnol.* **2012**, *23*, 236–242. [CrossRef]
13. Averill, C.; Finzi, A. Plant regulation of microbial enzymes production in situ. *Soil Biol. Biochem.* **2011**, *43*, 2457–2460. [CrossRef]
14. del Pozo, A.; del Canto, P. *Áreas Agroclimáticas y Sistemas Productivos en la VII y VIII Regiones*; Centro Regional de Investigación Quilamapu: Chillán, Chile, 1999.

15. Jackson, M.L. *Soil Chemical Analysis*; Prentice-Hall: Englewood Cliffs, NJ, USA, 1958; 498p.
16. Watanabe, F.S.; Olsen, S.R. Test of an Ascorbic Acid Method for Determining Phosphorus in Water and NaHCO3 Extracts from the Soil. *Soil Sci. Soc. Am. J.* **1965**, *29*, 677–678. [CrossRef]
17. Stockdalea, E.A.; Banningb, N.C.; Murphy, D.V. Rhizosphere effects on functional stability of microbial communities in conventional and organic soils following elevated temperature treatment. *Soil Biol. Biochem.* **2013**, *57*, 56–59. [CrossRef]
18. Anderson, J.P.E.; Domsch, K.H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* **1978**, *10*, 215–221. [CrossRef]
19. Garcia, C.; Hernandez, T.; Costa, F. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. *Commun. Soil Sci. Plant Anal.* **1997**, *28*, 123–134. [CrossRef]
20. Nannipieri, P.; Ceccanti, B.; Cervelli, S.; Matarese, E. Extraction of phosphatase, urease, proteases, organic-carbon, and nitrogen from soil. *Soil Sci. Soc. Am. J.* **1980**, *44*, 1011–1016. [CrossRef]
21. Tabatabai, M.A. Soil enzymes. In *Methods of Soil Analysis*; Page, A.L., Miller, E.M., Keeney, D.R., Eds.; ASA and SSSA Inc.: Madison, WI, USA, 1982; pp. 501–538.
22. Naseby, D.C.; Lynch, J.M. Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of Pseudomonas fluorescens on wheat seed. *Soil Biol. Biochem.* **1997**, *29*, 1353–1362. [CrossRef]
23. Tabatabai, M.A.; Bremner, J.M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *1*, 301–307. [CrossRef]
24. Sadzawka, A.; Grez, R.; Carrasco, M.; Mora, M. *Métodos de Análisis de Tejidos Vegetales, CNA Comisión de Normalización y Acreditación*; Sociedad Chilena de Ciencias del Suelo: Santiago, Chile, 2007.
25. Shade, A.; McManus, P.S.; Handelsman, J. Unexpected Diversity during Community Succession in the Apple Flower Microbiome. *Mbio* **2013**, *4*. [CrossRef]
26. Rilling, J.I.; Acuna, J.J.; Sadowsky, M.J.; Jorquera, M.A. Putative Nitrogen-Fixing Bacteria Associated With the Rhizosphere and Root Endosphere of Wheat Plants Grown in an Andisol From Southern Chile. *Front. Microbiol.* **2018**, *9*. [CrossRef]
27. Sakurai, M.; Wasaki, J.; Tomizawa, Y.; Shinano, T.; Osaki, M. Analysis of bacterial communities on alkaline phosphatase genes in soil supplied with organic matter. *Soil Sci. Plant Nutr.* **2008**, *54*, 62–71. [CrossRef]
28. Fraser, T.D.; Lynch, D.H.; Gaiero, J.; Khosla, K.; Dunfield, K.E. Quantification of bacterial non-specific acid (phoC) and alkaline (phoD) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields. *Appl. Soil Ecol.* **2017**, *111*, 48–56. [CrossRef]
29. Li, Z.; Chang, S.; Ye, S.; Chen, M.; Lin, L.; Li, Y.; Li, S.; An, Q. Differentiation of 1-aminocyclopropane-1-carboxylate(ACC) deaminase from its homologs is the key foridentifying bacteria containing ACC deaminase. *FEMS Microbiol. Ecol.* **2015**, *91*. [CrossRef] [PubMed]
30. Mariotti, M.; Masoni, A.; Ercoli, L.; Arduini, I. Above- and below-ground competition between barley, wheat, lupin and vetch in a cereal and legume intercropping system. *Grass Forage Sci.* **2009**, *64*, 401–412. [CrossRef]
31. Martin-Guay, M.O.; Paquette, A.; Dupras, J.; Rivest, D. The new Green Revolution: Sustainable intensification of agriculture by intercropping. *Sci. Total Environ.* **2018**, *615*, 767–772. [CrossRef] [PubMed]
32. Hinsinger, P.; Betencourt, E.; Bernard, L.; Brauman, A.; Plassard, C.; Shen, J.; Tang, X.; Zhang, F. P for two, sharing a scarce resource: Soil phosphorus acquisition in the rhizosphere of intercropped species. *Plant Physiol.* **2011**, *156*, 1078–1086. [CrossRef] [PubMed]
33. Watt, M.; Evans, J.R. Linking development and determinacy with organic acid efflux from proteoid roots of white lupin grown with low phosphorus and ambient or elevated atmospheric CO_2 concentration. *Plant Physiol.* **1999**, *120*, 705–716. [CrossRef] [PubMed]
34. Schröder, D.; Köpke, U. Faba bean (Vicia faba L.) intercropped with oil crops—A strategy to enhance rooting density and to optimize nitrogen use and grain production? *Field Crop. Res.* **2012**, *135*, 74–81. [CrossRef]
35. Xia, H.Y.; Wang, Z.G.; Zhao, J.H.; Sun, J.H.; Bao, X.G.; Christie, P.; Zhang, F.S.; Li, L. Contribution of interspecific interactions and phosphorus application to sustainable and productive intercropping systems. *Field Crop. Res.* **2013**, *154*, 53–64. [CrossRef]
36. Schulze, J.; Temple, G.; Temple, S.J.; Beschow, H.; Vance, C.P. Nitrogen Fixation by White Lupin under Phosphorus Deficiency. *Ann. Bot.* **2006**, *98*, 731–740. [CrossRef]

37. Wang, Z.G.; Jin, X.; Bao, X.G.; Li, X.F.; Zhao, J.H.; Sun, J.H.; Christie, P.; Li, L. Intercropping Enhances Productivity and Maintains the Most Soil Fertility Properties Relative to Sole Cropping. *PLoS ONE* **2014**, *9*, e0113984. [CrossRef]
38. Fuentes-Ponce, M.; Moreno-Espíndola, I.P.; Sánchez-Rodríguez, L.M.; Ferrara-Guerrero, M.J.; López-Ordaz, R. Dehydrogenase and mycorrhizal colonization: Tools for monitoring agrosystem soil quality. *Appl. Soil Ecol.* **2016**, *100*, 144–153.
39. Mengual, C.; Roldan, A.; Caravaca, F.; Schoebitz, M. Advantages of inoculation with immobilized rhizobacteria versus amendment with olive-mill waste in the afforestation of a semiarid area with Pinus halepensis Mill. *Ecol. Eng.* **2014**, *73*, 1–8. [CrossRef]
40. Fu, Z.D.; Zhou, L.; Chen, P.; Du, Q.; Pang, T.; Song, C.; Wang, X.C.; Liu, W.G.; Yang, W.Y.; Yong, T.W. Effects of maize-soybean relay intercropping on crop nutrient uptake and soil bacterial community. *J. Integr. Agric.* **2019**, *18*, 2006–2018. [CrossRef]
41. Dick, W.A.; Cheng, L.; Wang, P. Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biol. Biochem.* **2000**, *32*, 1915–1919. [CrossRef]
42. Wasaki, J.; Omura, M.; Ando, M.; Shinano, T.; Osaki, M.; Tadano, T. Secreting portion of acid phosphatase in roots of lupin (*Lupinus albus* L.) and a key signal for the secretion from the roots. *Soil Sci. Plant Nutr.* **1999**, *45*, 937–945. [CrossRef]
43. Tang, X.Y.; Bernard, L.; Brauman, A.; Daufresne, T.; Deleporte, P.; Desclaux, D.; Souche, G.; Placella, S.A.; Hinsinger, P. Increase in microbial biomass and phosphorus availability in the rhizosphere of intercropped cereal and legumes under field conditions. *Soil Biol. Biochem.* **2014**, *75*, 86–93. [CrossRef]
44. Song, Y.N.; Zhang, F.S.; Marschner, P.; Fan, F.L.; Gao, H.M.; Bao, X.G.; Sun, J.H.; Li, L. Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and faba bean (*Vicia faba* L.). *Biol. Fert. Soils* **2007**, *43*, 565–574. [CrossRef]
45. Kunito, T.; Isomura, I.; Sumi, H.; Park, H.D.; Toda, H.; Otsuka, S.; Nagaoka, K.; Saeki, K.; Senoo, K. Aluminum and acidity suppress microbial activity and biomass in acidic forest soils. *Soil Biol. Biochem.* **2016**, *97*, 23–30. [CrossRef]
46. Mei, P.P.; Gui, L.G.; Wang, P.; Huang, J.C.; Long, H.Y.; Christie, P.; Li, L. Maize/faba bean intercropping with rhizobia inoculation enhances productivity and recovery of fertilizer P in a reclaimed desert soil. *Field Crop. Res.* **2012**, *130*, 19–27. [CrossRef]
47. Bedoussac, L.; Journet, E.P.; Hauggaard-Nielsen, H.; Naudin, C.; Corre-Hellou, G.; Jensen, E.S.; Prieur, L.; Justes, E. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agron. Sustain. Dev.* **2015**, *35*, 911–935. [CrossRef]
48. Li, Q.H.; Sun, J.H.; Wei, X.J.; Christie, P.; Zhang, F.S.; Li, L. Overyielding and interspecific interactions mediated by nitrogen fertilization in strip intercropping of maize with faba bean, wheat and barley. *Plant Soil* **2011**, *339*, 147–161. [CrossRef]
49. Ragot, S.A.; Kertesz, M.A.; Bunemann, E.K. phoD Alkaline Phosphatase Gene Diversity in Soil. *Appl. Environ. Microb.* **2015**, *81*, 7281–7289. [CrossRef] [PubMed]
50. Martínez-Viveros, O.; Jorquera, M.A.; Crowley, D.E.; Gajardo, G.; Mora, M.L. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* **2010**, *10*, 293–319. [CrossRef]
51. Patel, J.S.; Singh, A.; Singh, H.B.; Sarma, B.K. Plant genotype, microbial recruitment and nutritional security. *Front. Plan Sci.* **2015**, *6*, 608. [CrossRef] [PubMed]